Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(7):627-635

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Effect of *Cinnamomum cassia* essential oil on antioxidative status in Nickel exposed rats during the development period.

Kahloula Khaled, Adli Djalleleddine Houari, Tiresamina, Talatizi Mustafa and Slimani Miloud

Laboratory of Biotoxicology, Pharmacognosy and Biological recovery of plants, Department of Biology, Faculty of Sciences, University of Dr Moulay Taher, Saida, Algeria

---

ABSTRACT

The present work deals with the extraction of the essential oil of the Chinese cinnamon Cinnamomum cassia CEO that takes part on the investigation dealing with erythrocyte antioxidant power of Ni SO_4 exposed wistar rats during gestation and lactation periods. The extraction by hydrodistillation allowed obtaining an essential oil with a yield of 1%. Furthermore, the phytochemical screening of the crude extract of the plant highlighted the presence of tannins, resins, saponins, terpenoids and flavonoids. The CPG-SM qualitative and quantitative analysis of the CEO has revealed the dominance of cinnamaldehyde (66.54%) and Z-cinnamaldehyde (3.22%). However, the antioxidant activity of CEO essayed in vitro showed a scavenging activity of DPPH free radicals with an IC50 of 83 .54 mg / ml. Besides, the analysis of erythrocyte antioxidant status indicated that Ni significantly increased (p <0.05) the enzymatic activities of both catalase (CAT) and superoxyde dismutase (SOD) and decreased (p <0.05) the enzymatic activity of the glutathione peroxidase (GPx) and the no enzymatic activity of the reduced glutathione (GSH), by involving a dysfunction of the antioxidant defense system. In contrast, the administration of the CEO had contributed significantly on the improvement of enzymatic antioxidant defenses against free radicals resulted from Ni enhanced vulnerability by decreasing significantly (p<0.05) the significant overexpression of both CAT and SOD, and increasing significantly the underexpression (p<0.05) of both GPx and GSH.

Key words: Catalase, *Cinnamomum cassia*, Glutathione peroxidase, cinnamon essential oil, Nickel, Superoxyde dismutase and Reduced Glutathione.

INTRODUCTION

Nickel (Ni) is an abundant element in the earth's crust that can exist in various mineral forms**.** It is an ubiquitous trace metal element that can present in drinking water, ambient air and food **[1].** Various pathologies can occur following the Ni exposure which are cutaneous allergies**[2]**, respiratory tract cancer **[3]**and animal foetotoxicities. Moreover, it was demonstrated that the cancer induced by the Ni is mainly due to the reactive oxygen species' formation**[1].** It involves lipid peroxidation which is related to the production of free radicals altering the cell's antioxidant statute **[4,5-6].**

Otherwise, the Chinese cinnamon "*Cinnamomum cassia*" is a commonly used spice in the world. This condiment contains an essential oil with various chemicals such as cinnamaldehyde and cinnamic acid. Thus, it was demonstrated that derivatives of this plant offer beneficial effects to the body: antioxidant, anti inflammatory, antimicrobial, and anticarcinogenic properties. These chemicals can even reduce the rate of lipids and their peroxidation, and both cardiovascular and neurological diseases' risk **[7, 8].**

The aim of this study is to evaluate the in vivo relative effect of the natural antioxidant properties of the essential oil EO of the cinnamon "*Cinnamomum cassia*" after an oxidative stress induced by the Ni exposition during the development period.

EXPERIMENTAL SECTION

1.1. Preparation of the plant material:
Essential Oil of Cinnamon (EOC) was obtained by hydrodistillation: 50g of cinnamon bark powder was mixed with 200 ml of distilled water and then heated for 3h. The EOC was collected and stored at 4°C for further use**[9]**. The EOC is prepared as an injectable solution by suspending it in sterile double distilled water **[10]**. This solution at a required dose «0.1 ml / kg» was injected intraperitoneally **[9]**each day for 21 days.

1.2. Phytochemical screening of the plant:
A phytochemical screening is accomplished to identify cinnamon's phytochemicals.

1.2.1.Detection of tannins:
The test has been done according to **[11].** The identification of tannins is done by mixing 10 ml of the ethanolic extract of cinnamon (70%) with few drops of ferric chloride reagent (FeCl3) (1%). The presence of tannins is indicated by a blue color.

1.2.2.Detection of resins:
10ml of the ethanolic extract were mixed with 20ml of Hcl. The appearance of turbidity indicates the presence of resins in the extract **[12].**

Detection of coumarins:
5ml of the ethanolic extract were putted in a test tube and covered by a filter paper soaked by NaOH. After 10 min of incubation on a water bath, the filter paper was exposed to UV light. The presence of coumarins is indicated by a green bright yellow color **[13].**

1.2.3.Detection of saponins:
The appearance of foam after a rigorous agitation of the filtrate for 5 minutes indicates the presence of saponins on the ethanolic extract **[13].**

1.2.4.Detection of terpenoids:
A mixture of 1 ml of acetic anhydride and 2 ml of concentrated sulphuric acid were added to 1 ml of the ethanolic extract. The appearance of reddish brown color indicates the presence of terpenoids**[11].**

1.2.5.Detection of flavonoids:
This technique consists to prepare two solutions:
The solution A:5 ml of the cinnamon ethanolic extract.
The solution B: 5 ml of ethanolic solvent + 5 ml of KOH (50%).
The appearance of yellow color after mixing the two solutions indicates the presence of flavonoids **[14]**.

1.3. DPPH radical scavenging activity:
The antioxidant activity was evaluated by measuring the scavenging activity of the free radical 2,2-diphenyl-1-picrylhydrazyl « DPPH ». The test consists on mixing the DPPH of a purple color with antioxidant molecules in order to measure their abilities to reduce the diphenylpicryl-hydrazine of a yellow color. For that, a series of EOC concentrations going from 10mg /ml to 100mg /ml was tested. The reaction mixture contains 10 ml of an ethanolic solution of DPPH (0,004%) which was incubated previously during 30 min on the dark. After 30 min, the absorbance was measured at 517 nm**[15, 16]**. The results were expressed by inhibitory percentage **(I%).**Whereas**,** the IC 50 was determined graphically.

I%= [(Abs control – Abs sample)/ Abs control] x 100

IC 50: The required antioxidant concentration to reduce 50% of the initial concentration of oxidants.

1.4. Determination of the chemical composition of the EOC by CPG/SM:
The analytic study of the EOC was realized on the quality laboratory AFAK Oran with VARIAN CHROMPACK - CP 3900 Gas Chromatography by injecting 0.2 µl of the extract.

- The vector gas: the helium He with a debit of 0.3 ml/min and
- The column: VF5 capillary column with the dimensions (30 cm X 0.25mm) and 0.25mm of interior diameter.

The stationary phase:
o Nature: 5% phényl-polysinoxane and 95% de methyl.
o Thickness : 0.25 µm

The computation of the injection's initial column temperature is 70ºC during 2,50 min, then it is elevated with a rate of 15ºC/min at 255ºC during 20 min.
• The detector: Mass spectrophotometer (Saturne 20200) at 250ºC linked by a computer with an appropriate software and a NIST databank to identify the compounds.

1.5. Animals of experimentation:
The experiences are realized using Wistar albinos rats weighting 200 to 350 gr when arriving to the laboratory. The rats are grouped by 3 on Makrolon cages (L x l x H=40×25×18 cm) of 2 females VS 1 male, disposed on a ventilated animalery at 21°C ± 1°C. After their arrival, they are used at least after one week. Animals have an ad libitum access to food and water whereas an artificial illumination establish a day/night cycle (one day between 7 and 19h).

1.6. Experimental protocol:
1.6.1.Groups repartition:
At D0 of gestation, females are divided into two lots:

NiLot: containing animals receiving the nickel sulfate at 0.2% on a double distilled water from the first day of gestation to weaning (n=30 rats).

T lot: containing animals who receive water without Nickel sulfate.
➢ Just after the females' throw, the youth outcome population was divided on 4 lots:

The (T) LOT, belonging to the (T) groupdo not receive any treatment by EOC.

The (T+HE) LOT, belonging to the (T) group receiving the EOC solution intraperitoneally during 21 days.

The (Ni) LOT, belonging the (Ni) group, do not receiving an EOC treatment.

The (Ni+HE) LOT, belonging to the (Ni) group, receiving the EOC solution intraperitoneally during 21 days.

1.6.2.Blood sampling:
At the end of treatment, rats have been sacrificed after one night's diet by decapitation after being intraperitoneally injected by chloral solution ($C_2H_3Cl_3O_2$) «10% » using 4mg/Kg body weight. Blood has been recuperated on EDTA tubes for enzymatic essays.

1.6.2.1. Preparation of erythrocytes:
After recuperating blood samples that have been centrifuged at 3000 tr/min during 15 min, the serum has been isolated and the remaining pellet containing erythrocytes has been washed three times with a saline solution at 9 g/l, then cells lysis has been performed by adding icy distilled water and incubated during 15 min on the ice. The lysate is then recuperated after centrifugation at 5000tr/min for 5min to serve for the antioxidant enzymes essay marking the oxidant statute **[17].**

1.6.3.In vivo antioxidant activity:
1.6.3.1. Catalase (CAT EC 1.11.1.6) :
The CAT activity has been analyzed according to the method of **[18].** The reaction mixture contains 1.0 ml of phosphate buffer, 0.1 ml of the sample and 0.4 ml of H2O2. The reaction arrest is done by adding 2 ml of dichromate acetic acid which is constituted of 5% of sodium dichromate and glacial acetic acid «1:3 (V/V)». The absorbance is determined at 570 nm.

1.6.3.2. Glutathionperoxydase (GSH-Px, EC 1.11.1.9):
The GSH-Px activity has been measured using the method of **[19].** The reaction consists to mix 0.2 ml of tris-Hcl buffer, 0.1 ml of sodium azid, 0.2 ml of the sample, 0.2 ml of glutathione and 0.1 ml of hydrogen peroxide. Then the mixture is incubated at 37°C for 10 min. The arrest of reaction has been accomplished by adding 0.4 ml of trichloroacetic acid TCA «10%». After that, the mixture is submitted to a centrifugation of which the supernatant serving to determine the rate of glutathione is mixed with Ellman reagent (19.8 mg of 5, 5'-dithiobisnitro benzoïque acid (DTNB) in 100 ml de solution de nitrate de sodium à 0.1%).

1.6.3.3. Superoxydedismutase SOD :
Erythrocyte superoxydedismutase SOD has been determined according to the method of **[20].** The reaction mixture contains 0.2 ml of Ethylene Diamine Tetraacetic Acid (EDTA) at 0.1 M, 0.1 ml Nitrobluetetrazolium (NBT) at 1.5 mM, 1.2 ml of potassium phosphate buffer at 0.067 M, and 0.5 of the enzymatic extract. The mixture is then incubated for 5-8 minutes. The lecture is then done at 560 nm.

1.6.3.4. Reduced glutathione:
Reduced glutathione (GSH) has been analyzed using the method of **[21].** that consists to mix 0.5 ml of the sample and 1.0 ml of sulfosalicylic acid (4%). The samples have been maintained at 4°C for one hour then centrifuged at 1200 tr/min for 15 min. The analysis mixture contains 0.1 ml of supernatant and 2.7 ml of phosphate buffer. The lecture is done immediately at 412 nm.

1.7. Statistical analysis:
Each assay was performed in duplicate, and data were expressed as mean±S.E.M. The statistical significance of the differences between the groups was calculated using Student's t-test for independent means. Two-tailed values of $P < 0.05$ were considered significant.

RESULTS

3.1. Yield of EOC:
EOC has been obtained with a yield of 1% regarding to the total quantity of the dried material.

3.2. Phytochemical screening of the plant:
Phytochemical tests indicated the positive presence of the tested compounds.

3.3. DPPH radical scavenging activity:
The antioxidant activity of the ethanolic extract of Chinese cinnamon vis-a-vis the free radical DPPH has been evaluated using a spectrophotometer following its reduction which is accompanied by its passage from the purple color (DPPH•) to the yellow one (DPPH-H) which is measurable at 515nm. This reduction ability is determined by the decrease in absorbance which is induced by antiradicals.

The antioxidant activity results reveal after calculating the IC50 a concentration of 83.54 mg/ml.

3.4. Detected chemicals by CPG/MS :
The analysis of EOC by gas chromatography permitted to identify 14 major compounds cited according to the elution order (**Table01**). 14 compounds representing the percentages sum of the obtained compounds has been identified representing 0.78 % of phenols, 1.27% of hydrocarbons, 3.878% of terpenes and 4.077% of terpenic alcohols. The major compounds of this oil are: E-cinnamaldehyde (66.54%) and Z-cinnamaldehyde(3.22%).

Table 01.Concentration and retention time of the different obtained compounds by Gas chromatography analysis of the essential oil of *Cinnamomum cassia*

Compound	Time Retention (min)	Concentration (%)
α-pinene	10.70 min	0.395
β-pinene	10.80 min	0.109
β-Phellandrene	6.121 min	1.19
α-*Phellandrene*	7.29 min	0.080
Camphene	12.55 min	0.184
Limonene	9.81 min	1.189
γ-terpinene	10.10 min	0.291
Chavical	21.77 min	0.30
Linalol	15.10 min	3.707
p-cymene	06.68 min	1.71
Terpiène-4-ol	16.70 min	0.37
E-cinnamaldehyde	22.26 min	66.54
Eugenol	24.01 min	0.48
Z-cinnamaldehyde	22.60 min	3.22

3.5. In vivo antioxidant activity:
3.5.1.The CAT activity :
The results of the figure 01 relative to the erythrocyte CAT activity show that this enzyme is overexpressed in intoxicated rats compared with control rats. However intoxicated rats administration of EOC has reduced

significantly ($p<0.05$) the enzymatic activity of the CAT compared with non treated rats. Similarly, this significant decrease ($p<0.05$) of activity has been observed in EOC treated control rats compared with no treated control rats.

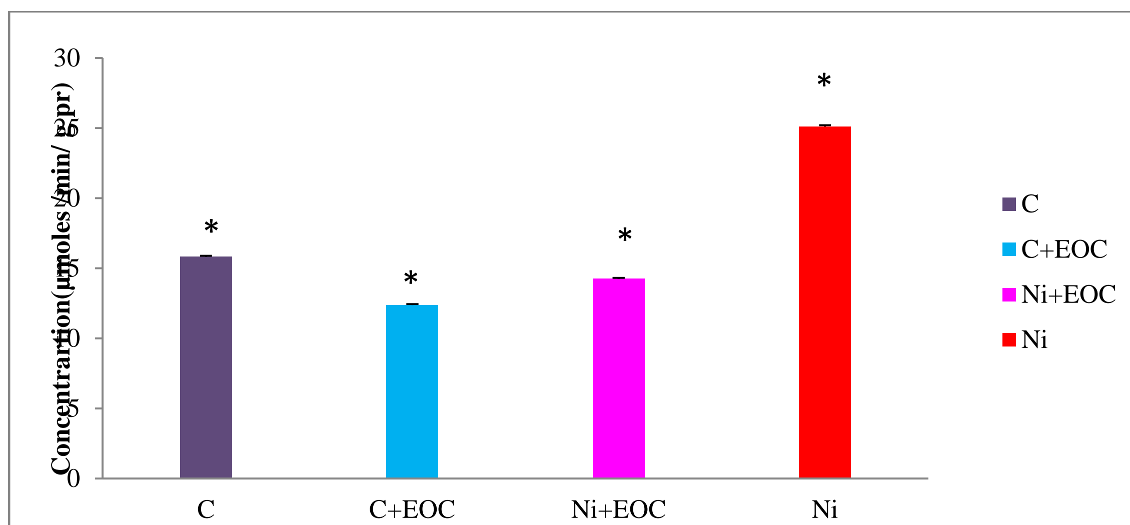

Figure 1: The effect of the EOCon the catalase enzyme activity of erythrocyte in control and exposed Ni rats (*: p <0.05)

3.5.2.The GPx activity:

Glutathione peroxidase constitutes one of the most important enzymatic systems of protection which insure the detoxification of hydrogen peroxide under normal physiological conditions. The obtained results, relative to the GPx action indicate that it exists a significant decrease of the GPx enzymatic activity in Ni exposed animals compared to control ones. However, statistic tests present a significant increase ($p<0.05$) of this activity in EOC treated Ni rats compared with the no treated ones (Figure 02).

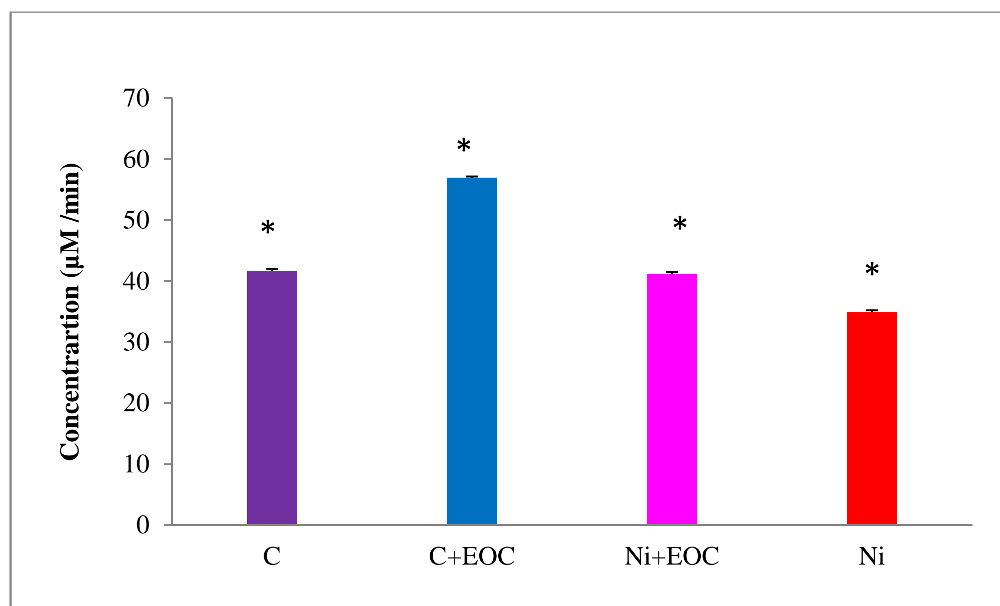

Figure 2: The effect of the EOC on the Glutathion Peroxydase enzyme activity of erythrocyte in control and exposed Ni rats (*: p <0.05)

3.5.3.GSH activity :

According to the GSH erythrocyte activity (Figure 03), we noticed the presence of significant decrease ($p<0.05$) of the activity of this flavoprotein in Ni intoxicated rats, an increase ($p<0.05$) of activity in EOC treated rats and a significant decrease of activity ($p<0.05$) in EOC treated control rats compared to the non-treated ones.

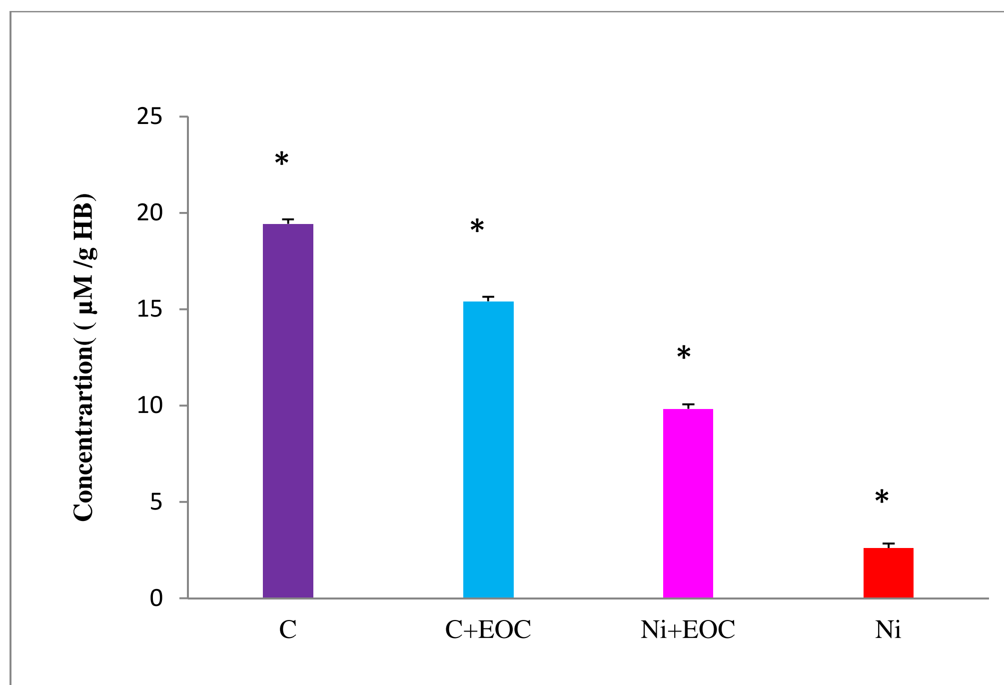

Figure 3: The effect of the EOC on reduced glutathione in erythrocyte control and exposed Ni rats (*: p <0.05)

3.5.4.SOD activity :

Superoxyddismutases (SOD) are able to eliminate the superoxide anion by producing two molecules: oxygen and hydrogen peroxide. The combined results (Figure 04) indicate a significant SOD overexpression ($p<0.05$) in rats having been exposed to Ni during the development period compared to control ones. Whereas a significant decrease ($p<0.05$) of SOD activity is observed under the EOC action in intoxicated rats. However, a significant decrease ($p<0.05$) of this enzyme expression in EOC treated control rats has been notices.

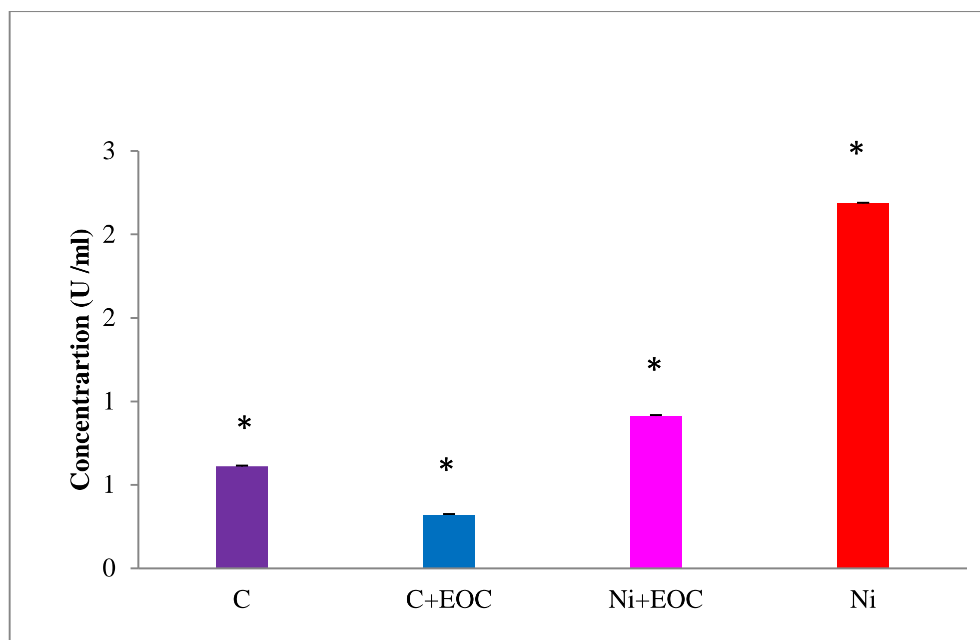

Figure 4: The effect of the EOC on the superoxide dismutase enzyme activity of erythrocyte in control and exposed Ni rats (*: p <0.05)

DISCUSSION

The EO of *Cinnamomum cassia* has been obtained with hydrodistillation with 1% yield, this is in accordance with research works of **[22,23]** who reported yields comprised between (0.4-4.9%).

After the accomplishment of a preliminary phytochemical screening of the plant, we could revealed the presence of tannins, resins, coumarins, saponins, terpenoids and flavonoids. Our results are in accordance with research works of**[24]** who revealed in addition to these compounds other chemicals such as: alkaloids, polyphenols, carotenoids, ascorbic acid, thiamine, riboflavins and niacins with the dominance of polyphenols, carotenoids, flavonoids, and ascorbic acid.

However, our EOC exercised a potential antioxidant activity at 59.85%. Whereas,**[24]** had posted a reduction of86.66 ± 4.33 % after testing the crushed cinnamon. On the same context, the value of IC 50 was 83.54 mg/ml and is not consistent with the one revealed by **[25]** who reported a value of10.090 mg/ml after testing an EOC extracted by the method of supercritical fluid.

Oxidative stress is defined as a pronounced disequilibrium between antioxidant and oxidant elements in favor of oxidants et their deleterious effects. The oxidative stress origins are multiple and result essentially after the formation of Reactive Oxygen Species on organism **[26].**

Otherwise, the toxicity of Nickel Sulphate has been evaluated by the essay of erythrocyte antioxidant enzymes such as CAT, GPx, SOD and a non enzymatic compound which is the GSH. Whereas, These antioxidant enzymes permit to maintain the REDOX potential homeostasis**[27].**

The relative results to the CAT activity reveal a significant increase of its activity in control rats. This result is in accordance with what had been identified by**[28]** who was attributed to this increase as a result of increase of the blood Ni rate with an overexpression of the CAT. Thus,**[29]** were brought similar results after assaying the activity on adult rats' red blood cells which were intraperitoneally received the $NiSO_4$ and **[30]** have noted this overexpression on human lymphocytes. Otherwise, this is clearly disagreed with the researches of **[31]** who have notices a decrease of the CAT activity at the hepatic level and with those of**[32]** who have induced an oxidative stress state by $NiCl_2$ in chickens.

The CAT could partially reduce the oxidants formation resulting from the rate of Ni elevation. Thus It prevents the generation of hydroxyl radicals **[31].**

On the same context, it was remarked that in the period of development, rats Ni oral administration had provoked a significant decrease of the glutathione peroxidase (GPx) activity. These results are consistent with those of**[31, 32]** who had posted that the Ni administration can cause a decrease of the GPx activity. However, **[33]** has revealed that this activity decrease can conduct to an oxidative stress state on the red blood cells.

Furthermore, the obtained results of the non enzymatic activity « GSH » has revealed a significant decrease of its rate in Ni exposed rats. This is consistent with the researches of **[29,31,32].** In fact, **[33]** has suggested that the GSH activity exhaustion is explained by the increase of free radicals which are toxic metabolites requiring eventually a detoxification by the scavenging, thus, this decrease can be explained by the fact of GSH participation on the ROS and metallic traces detoxification reactions. Whereas, according to **[34]**, this decrease enhanced the cell oxidative stress sensibility after the overformation of ROS.

[31] had shown that the Ni induced lipid peroxidation is fought essentially by the GSH. However the research works of **[4]** had confirmed that the decrease of the GSH activity that elevates free radicals damages probabilities is directly caused by the rise of rate of malonic dialdehyde (MDA) that could be linked with the GSH.

Concerning the SOD essay, we had remarked that Ni intoxication had significantly increased the concentration of this enzyme in erythrocytes. This is in accordance with the research works of**[26]** who had suggested that The Ni play an important role as a SOD catalyst. Similarly, **[29]** had obtained similar results testing erythrocytes of adult rats receiving a $NiSO_4$ solution intraperitoneally.

Our results have shown that the chronic treatment by the Ni had provoked important oxidant damages on erythrocytes conducting at first to lipid peroxidation which habitually accompanied by a significant increase of free radicals rate, then, this give naissance de a series of biochemical changes interfering on the enzymatic antioxidant activity. However, these cell disturbances were provoked by the loss of the ability of fight against the damages which are caused by free radicals attack. This was confirmed by the research works of **[33]**.

In fact,**[35]** suggested that Ni as one of the heavy metals that presents a high affinity with sulfhydryl groups (-SH), it permit easily the activation of antioxidants containing the sulphur.

It had been demonstrated that the peroxidative effect of Ni can be the result of the Nickel-MDA correlation **[4].** When the antioxidant defenses are weakened or exceeded oxidative stress can provoke an enzymatic inactivation and a lipid peroxidation **[36]** the fact that in all xenobiotics aggressions, the protein metabolism is modified to produce defense systems **[33]**.

Furthermore, the post EOC treatment essays in Ni intoxicated rats had revealed that both CAT andGPx activities are merely closed to those observed in control rats. EOC seems to have a effect vis –a-vis the SOD and GSH biomarkers.

This detoxification is envisaged by inducing the production of de glutathion-S-transferase**[37].** Whereas, other authors have shown that cinnamaldehyde, eugenol and linalool are responsible for the benefic effect against lipid peroxidation **[8].**

Furthermore, **[38]** had in vitro essayed analternative SOD antioxidant activity consisting essentially on scavenging the superoxide free radical after an EOC exhibition. Whereas, **[8]** have reported that EOC is implicated the maintain of the SOD homeostasis.

On the same context,**[27]** have demonstrated that EOC and its major compound cinnamaldehyde have the ability to repress the oxidative stress and lipid peroxidation due to their interference on the improvement of GSH, CAT and GPx activities which permit to reduce the rate of ROS. Whereas, **[39]** had reported that the cinnamaldehyde is the responsible compound that modify the rate of antioxidant enzymes resulting from an oxidative stress.

CONCLUSION

The Ni intoxication during the period of development had caused a dysfunction of the oxidative status on erythrocytes which is the consequence of the disturbance of the activities GPx, GSH, CAT and SOD. Whereas, the EOC treatment had permitted to significantly improve the tested activities and establish the oxidative status.

REFERENCES

[1] M Cempel ; G Nikel.Nickel.*Polish J. of Environ. Stud.*,**2006,**15,375-382.
[2] A Cavani. *Toxicol*, **2005**, 209, 119-121.
[3] KS Kasprzak; FW JrSunderman; K Salnikow. *Mutat Res*., **2003**, 533, 67–97.
[4] N Hfaiedh ; MS Allagui ; FCroute ; JP Soleilhavoup ; F Guermaz ; A Kammoun ; A El Feki. *Andrologie*.,**2004**, 14, 443-451.
[5] KK Das;SN Das; SA Dhundasi.*The Indian Journal of Medical Research*.,**2008**, 128, 412–25.
[6] MH Ibrahim, HZE Jaafar; Karimi E; A Ghasemzadeh.*Sci. World J*.,**2014**, 2014, 1-13.
[7] Y Shen; LN Jia ; N Honma ; T Hosono; T Ariga ; T Seki. *Journal of Traditional and Complementary Medicine*.,**2012**, 2, 27-32.
[8] PVRao;SH Gan.*Altern.Med*.,**2014**, 2014.
[9] S Li; C Wang; X Peng; X Wu. *Journal of Food and Nutrition Research*.,**2013**, 12, 7–12.
[10] S Halder; AK Mehta; R Kar; M Mustafa; PK Mediratta; KK Sharma. *Planta Med*, **2011**, **77**, 830–834.
[11] OA Aiyegoro; AI Okoh.*BMC Complement.Altern.Med*., **2010**, 10, 21.
[12] MRAl-Balany. Effect of crude plant Extracts and Vasicine alkaloid of AdhatoaVasica in some pathogenic microorganisms, Msc.Thesis, faculty of Science, Baghdad University, Iraq, **2004**.
[13] H Farhan; H Rammal; AHijazi; H Hamad; B Badran. *Ann. Biol. Res.*, **2012**. 3, 149–156.
[14] H Farhan; H Rammal; AHijazi; H Hamad; B Badran. *Int J Curr Pharm Res.*, **2012,**4,1–5.
[15] A Bouguerra. Etude des activités biologiques de l'huile essentielle extraite des graines de *Foeniculumvulgare* Mill. en vue de son utilisation comme conservateur alimentaire. Doctorat thesis, Institut de la nutrition, de l'alimentation et des technologies agro-alimentaires, MentouriUniversity, Constantine, Algéria, **2012**.
[16] L Imène. *Nature & Technologie*., **2012**, 7, 44–52.
[17] L Hocine. Détermination des marqueurs du stress oxydatif chez la progéniturede rats « Wistar » obèses sous régime hypergras. Magister thesis, Faculty of sciences, AbouBekrBelkaid.University, Tlemcen.Algérie, **2011**.
[18] AK Sinha. *Analytical Biochemistry*.,**1972**, 47, 389-394.
[19] JT Rotruck; AL Pope; HE Ganther; AB Swanson; DG Hafeman; HG Hoekstra. *Science*.,**1973**, 179, 558-590.
[20] CC Winterbourne; RE Hawkins; M Brain; RW Carrel.*J. Lab.chem.Med*., **1975**, 85, 337-341.
[21] D.JJollow ; JR Mitchell ; N Zampaglione; JR Gillette. *Pharmacology*.,**1974**, 11, 151-169.
[22] B Krishnamoorthy; JT Zachariah; J Rema; PA Mathew. *Journal of Spices and Aromatic Crops.*,**1999**, 8, 93-195.
[23] L Wei; Y Zhang; B Jiang.*Adv. J. Food Sci. Technol.*, **2013**, 5, 1436–1442.

[24] U Kumar;V Prakash. *Int J Pharm Pharm Sci.*, **2012**, 4, 291-295.
[25] CH Yang; RX Li; LY Chuang.*Molecules*.,**2012**, 17, 7294–7304.
[26] B De Moffarts ; N Kirschvink ; J Pincemail ; P. Lekeux. *Ann. Med. Vet.*, **2005**, 149, 1–9.
[27] ST Chou; WL Chang; CT Chang; SL Hsu; YC Lin; Y Shih. *Int. J. Mol. Sci.*, **2013**, 14, 19186–19201.
[28] MC Martín Mateo; B Martín; M Santos Beneit; J Rabadán.*Biol. Trace Elem. Res.*, **1997**, 57, 79–90.
[29] KK Das; A Das Gupta; SA Dhundasi; A M Patil; SN Das; JG Ambekar. *BioMetals*.,**2007**, 20, 177–184.
[30] CYChen; YF Wang; YH Lin; SF Yen.*Arch Toxicol*.,**2003**, 77, 123-130.
[31] KKDas; SN Das; SA Dhundasi.*The Indian Journal of Medical Research*.,**2008**, 128, 412–25.
[32] B Wu; H Cui; X Peng; J Fang; Z Zuo; J Deng; J Huang. *Health*.,**2013**, 5, 454–459.
[33] S Saka ; A Bahi ; W Aouacheri. *Ann. Toxicol. Anal.*, **2011**, 23, 3, 139–145.
[34] NAuberval ; S Dal-Ros ; V Schini-Kerth; M Pinget; S Sigrist. *Diab.Metab*., 2010, 36, A79.
[35] Rioux, C. Stress oxydatif et prévention des maladies chroniques la supplémentation s'impose-t-elle. Mémoire de maîtrise en kinésiologie pour l'obtention du grade de maître en sciences, Faculté des études supérieures dans le cadre du programme (M.Sc.), Laval university, **2009**.
[36] M Melila ; W Poutouli ; K Amouzou ; G Tchangbedji ; MTchaou, A Doh ; C Goto.*Int. J. Biol. Chem. Sci.*, **2012**, 6, 1263–1270.
[37] SAhmed. *Hum. Exp. Toxicol*., **1999**, 18, 691–698.
[38] JC Liao; JS Deng; CS Chiu; WC Hou; SS Huang; PH Shie; GJ Huang. *Evidence-based Complement. Altern.Med.*, **2012**, 2012, 429320.
[39] CL Wu; HT Chang; YR Hsui; YW Hsu; JY Liu; SY Wang; ST Chang.*BioResources*.,**2013**,8, 571–580.